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Effect of storage temperature on the survival or growth of Listeria monocytogenes on whole and fresh-cut produce

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EFFECT OF STORAGE TEMPERATURE ON THE SURVIVAL OR GROWTH OF *Listeria monocytogenes* ON WHOLE AND FRESH-CUT PRODUCE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Whole and fresh-cut produce are minimally processed and, therefore susceptible to microbial contamination. This study examined the survival or growth of *Listeria monocytogenes* on whole, and fresh-cut produce at different storage temperatures. Fresh fruits (cantaloupes, pears, pineapples, papayas, and watermelon) and vegetables (broccoli, cauliflower, lettuce, kale, and green bell peppers) were cut into 25 g pieces and were spot inoculated with 0.5 mL (8 Log CFU/mL) of *Listeria monocytogenes.* Inoculated fresh-cut samples were stored at 4°C or 13°C for 6 days. To represent the outer surface of the produce, cantaloupes and green bell pepper disks (20 cm^2) were cut with the rind and spot inoculated on the rind part with 0.5 mL of inoculum and were stored at 24°C for 8 days or 4°C for 14 days respectively. *Listeria* count on all fresh-cut samples except broccoli and cauliflower remained similar throughout the storage time at 4°C. At 13°C, *Listeria* counts increased significantly ($p<0.05$) within one day on fresh-cut watermelon (1.1 Log CFU/g) and cantaloupe (1.52 Log CFU/g). Similar results were observed on fresh-cut pear (1.0 Log CFU/g), papaya (1.65 Log CFU/g), and green bell pepper (1.2 Log CFU/g) after two days at 13°C. Pineapple samples did not favor the growth of *Listeria*, and a reduction of >1 log was observed at both storage conditions. *Listeria* levels significantly increased in fresh-cut lettuce 13°C but remained stable on kale, cauliflower, and broccoli. Listeria growth was not favored on rind samples with levels remaining stable on cantaloupe rind (8 days at 24°C) and was below the detectable limit of the test on bell pepper after 14 days of storage at 4°C. The results obtained during this experiment serve to establish *Listeria monocytogenes* ability to survive in the fresh-cut produce stored at 4°C, as well as the favorable growth environment created in these surfaces at 13°C.

1. INTRODUCTION

Fresh and fresh-cut produce are highly susceptible to microbial contamination due to pre-harvest and post-harvest activities. This commodity comes from agricultural environments and frequently ends up being exposed to sources of contamination, such as irrigation water, animals, and soil (Allende et al., 2015; Amoah et al., 2015). Contaminated produce may further contaminate processing and packaging facilities resulting in long-term food safety risks in food industries (Aceituno et al., 2017; Dalmasso et al., 2015).

Listeria monocytogenes, a member of the genus *Listeria*, has been well recognized as a foodborne pathogen and commonly found and isolated from fresh produce (Berche et al., 2001; Goldfine & Shen, 2007). Once *L. monocytogenes* enter into the processing facilities, it can survive for long periods due to favorable humidity and temperature (Dalmasso et al., 2015). Several outbreaks were reported, one of them lasting from 2015 to 2016, related to *Listeriosis* caused by packaged salads produced by Dole. This outbreak was traced back to a single processing facility in Ohio (CDC, 2016).

Microbial risk in fresh produce begins at an early stage of production, the pre-harvest stage (Barton, Bennett, Hill, Littrell, & Mahovic, 2015). Pathogens initially attach to the surface of produce, however during slicing pathogens may transfer to the flesh. Produce is rich in nutrients that can support the growth of pathogens (J. Huang, Luo, Nou, Zheng, & Zhou, 2019). Microbial attachment at first is reversible. The pathogens in this state can be easily removed by mechanical means. Once biofilm formation occurs this attachment becomes irreversible, only being removed by physical or chemical energy. Irreversible attachment is aided by the type of flagella the bacteria has (Fletcher, Flint, Kyere, Palmer, & Wargent, 2018).

Minimally processed produce depend mostly on natural barriers such as peels and rinds to protect from bacteria infecting the inner mesocarp. Slicing or cutting increases the risk of microbial contamination and proliferation due to available nutrients (Bortolossi et al., 2016; Kumar, Shafiq, & Yousuf, 2015). These conditions, along with its ability to grow at refrigeration temperatures, aid *L. monocytogenes* in attaching to fresh-cut produce (East et al., 2016). Once the pathogen is able to survive on the surface of produce, biofilms are formed, and it becomes difficult to eliminate the bacteria. *L. monocytogenes* biofilm formation is dependent on environmental conditions and populations (Barbuddhe et al., 2015; Briandet, Brisse, Desvaux, Guilbaud, & Piveteau, 2015).

Cross-contamination may occur at several points during post-harvest conditioning. As stated by Dalmasso and colleagues (2015), food-processing environments are affected by three types of possible contamination. The first being sporadic contamination, which occurs mainly during contact between unclean and clean surfaces. This occurs mostly during the receiving of raw materials from the field, which have a greater potential of carrying microbial pathogens. Another possible contamination scenario is hotspots of contamination; this refers to areas in which improper cleaning has led to build-up of waste and in many cases, biofilm formation of *L. monocytogenes*. These hotspots are difficult to identify. Due to their high microbial population, there becomes a high risk of produce contamination. The final scenario is widespread contamination, caused mainly by improper cleaning protocols, which allow isolated contaminations to spread throughout most of the food-processing environment (Dalmasso et al., 2015). In order to reduce risk of contamination during post-harvest processing it is necessary to apply proper sanitizing procedures for the produce, as well as proper and constant sanitizing protocols for the entire food-processing facility, as to minimize the possibility that the environment becomes a vector of contamination (Allende et al., 2015).

L. monocytogenes is a psychrotrophic bacteria, capable of growing at 30-37°C, as well as under refrigeration temperatures. This foodborne pathogen produces a sickness known as Listeriosis; the main virulent serotypes able to produce Listeriosis are 1/2a, 1/2b, and 4b (Goldfine & Shen, 2007). The infectious dose of *L. monocytogenes* that may cause Listeriosis remains unclear. However, the estimated concentration is as high as 8 or 7 Log CFU/g. There have been some cases of Listeriosis caused by concentrations around 2 or 4 Log CFU/g in immunocompromised individuals (Berche et al., 2001; USDA, 2003). The population most susceptible are the elderly, pregnant women, infants, and individuals with deficient immune systems (USDA, 2003). *L. monocytogenes* is commonly found in produce due to its presence in soil and water. However, the diversity of agricultural practices applied to different fruits and vegetables, as well as different surface characteristics and available nutrients make it difficult to accurately understand the survival or growth potential of this pathogen.

Moreover, most studies that seek to determine the die-off rates of *L. monocytogenes* use inoculum levels as high as 8 Log CFU/g; however, most contaminations occur at lower concentrations. Haven taken into consideration lack of this information, this study determined the survival and growth of *L. monocytogenes* on the surface of fresh-cut fruit and vegetables during different storage conditions. The study also determined the survival and growth of *L. monocytogenes* on the external surfaces of cantaloupe and green bell peppers during different storage conditions.

2. LITERATURE REVIEW

2.1. Fresh produce safety issues

Fresh produce are commonly part of human diets due to their healthy nature. They are a significant source of vitamins, minerals, and fiber (Rickman, Barrett, & Bruhn, 2007). The regular consumption of these products, along with maintaining a healthy lifestyle, such as frequent exercise has been found to protect from chronic diseases (Qadri, Yousuf, Srivastava, & Yildiz, 2015; Rickman et al., 2007). These benefits coupled with the promotion of healthy diets has led to a constant increase in the consumption of fresh produce over the past decades (Warriner, Huber, Namvar, Fan, & Dunfield, 2009). Produce is mainly seasonal and also commonly grown in specific areas in the world, because of this produce is frequently imported or stored for prolonged periods after harvest. These scenarios can lead to the propagation of bacteria that contaminates the produce. Considering this there has also been an increase in outbreaks related to fresh produce (Y. Huang & Chen, 2011; Olaimat & Holley, 2012; Rickman et al., 2007).

Increased demand of fruits and vegetables has led to an increase in imports to the United States, with more producers trying to get into the market in order to fulfill the market´s demand for off-season produce. Government agencies in charge of food safety continue struggling to control imported produce that is determined to be contaminated. Domestic crops are also a major issue, with most of the fresh produce being designated to consumer markets without kill steps able to control and reduce microbial contamination. Many large outbreaks have been related to regularly

consumed commodities such as apples, cantaloupes, raspberries, lettuce, spinach, tomatoes, and sprouts (Gerba, Matthews, & Sapers, 2014).

The majority of fresh produce are typically consumed raw or minimally processed and there is a lack of efficient methods of controlling bacteria on these surfaces. The high risk, makes prevention the best option when dealing with pathogens. Produce is estimated to be responsible for 20 million illnesses, resulting in a loss of $38.6 billion annually in the US (Schariff, 2010). Between 1973–2014, fruit and vegetable crops were the most commonly implicated commodities for several foodborne illness outbreaks (Crowe, Mahon, Vieira, & Gould, 2015; Nguyen et al., 2015). The common etiological agents haven been viruses (norovirus and hepatitis A); protozoa; shiga toxin–producing *Escherichia coli, Listeria monocytogenes, Vibrio cholera,; Aeromans hydrophila, Salmonella, Bacillus cereus* and *Campylobacter* (Crowe et al., 2015; M. D. Danyluk, Goodrich-Schneider, Schneider, Harris, & Worobo, 2012; Herman, Hall, & Gould, 2015; Keskinen, Burke, & Annous, 2009). Salmonella and E. coli O157:H7 were the etiological agents associated with most of the outbreaks illnesses (Buck, Walcott, & Beuchat, 2003; Warriner et al., 2009).

2.2. Sources of contamination

2.1.1. Food safety risks: potential on-farm sources of contamination

Several studies have traced pre-harvest events as important causes of microbial contamination (Park et al., 2012). Fruit and vegetable crops have the potential to be contaminated with pathogenic microorganisms in the field. Pre-harvest contamination of produce commonly originates from the soil, inadequately composted manure, contaminated irrigation water, and

improper human handling of produce (Annous, Solomon, Cooke, & Burke, 2005; Tomas-Callejas et al., 2011). The intrusion of crops by wild animals, birds, reptiles, and rodents, as well as insects and nematodes, act as vectors for transferring various pathogens (Brandl, 2006). Contamination sources and pathways are discussed below.

Manure is an important source of pre-harvest contamination of fresh produce. The manure obtained from livestock may be contaminated with enteric pathogens such as *E. coli* O157 and *Salmonella* spp. (Doyle & Erickson, 2008). *C. jejuni* is a normal member of the gastrointestinal microflora of poultry, pigs, and cattle (Warriner et al., 2009). In the agricultural field, foodborne pathogens can survive in animal manure for extended periods resulting in fresh produce contamination. *E. coli* O157:H7 survived in bovine manure for over 70 days at 5^{o}C (Semenov, van Bruggen, van Overbeek, Termorshuizen, & Semenov, 2007). At 22 or 30^{o}C, this pathogen survived up to 49 days (Semenov et al., 2007). Nicholson, Groves & Chambers, (2005) observed that, following manure spreading to land, *E. coli* O157, *Salmonella*, and *Campylobacter* survived in the soil for up to one month, and *Listeria* survived for more than one month. The contamination of fresh produce may occur by subsurface runoff, splash dispersal during rain events and irrigation, dust particles transfer from soil onto produce surfaces and during pre-harvest activities (Liu, Hofstra, & Franz, 2013; Nicholson, Groves, & Chambers, 2005).

Soil is a source of several human pathogens, including *B. cereus*, *Cl. perfringens*, *Cl. botulinum*, *L. monocytogenes* and *Aeromonas* (Beuchat, 1995). The types of pathogens present in the soil may be dependent on the manure type, management during stockpiling, method of application, application rate, frequency of application, and time between application and planting or harvesting (Whipps, Hand, Pink, & Bending, 2008). In addition, contaminated irrigation water may result in soil contamination (Liu et al., 2013). The level of contamination of fresh produce is

influenced by factors such as proximity of the edible portion of the plant to the soil, concentration of pathogens in contaminated soil and the type of crop grown in the soil (Doyle & Erickson, 2008). Root crops are more likely to be contaminated than crops, which grow above the ground. While greater microbial populations in soil pose greater risk of contamination of crops (Liu et al., 2013).

Studies have reported the internalization of pathogens in plants from soil by root uptake (Franz et al., 2007). Significant populations of both *S. enterica* serovar Typhimurium and *E. coli* O157:H7 was observed in sterilized leaf samples from plants grown in contaminated soil (Franz et al., 2007). The common route of internalization of human pathogens has been proposed to be penetrations at cracks in seed coats, invasion at lateral root junctions in seedlings, and aerial tissues (Doyle & Erickson, 2008).

Irrigation water is a potential vehicle of human pathogens for pre-harvest contamination of produce. The level of produce contamination is greatly influenced by the quality of irrigation water and type of irrigation system (Aruscavage, Lee, Miller, & LeJeune, 2006; Brackett, 1998; Warriner et al., 2009). The risk associated with irrigation water is highlighted in the Food Safety Modernization Act Produce Safety Rule (FSMA PSR), and it's the first time we have a federal regulation specific for farm food safety. According to the rule, for water applied to products that are consumed raw with a direct application method the geometric mean (GM) and the statistical threshold (STV) of generic *E. coli* should not exceed 126 CFU/100 mL and 410 CFU/100 mL of water, respectively (FDA, 2015). Although all kinds of irrigation systems pose risks of produce contamination, flood and spray irrigation poses a greater risk of disseminating pathogens on produce surfaces. This is due to the wider range covered by this kind of irrigation, which means contaminants can come in contact more frequently with harvestable parts of crops (Olaimat & Holley, 2012). They can deliver microbial pathogens onto the edible portion of the crop indicating

a greater risk of contamination (FDA, 1998). (Solomon, Potenski, & Matthews, 2002) reported that spray irrigation resulted in the contamination of 90% of lettuce plants with *E. coli* O157 compared to 19% of lettuce plants contaminated by surface irrigation. Some studies revealed that sprinkling systems increased the likelihood of internalizing pathogens in produce (Alegbeleye, Singleton, & Sant'Ana, 2018). Although numerous studies have demonstrated the potential correlation between microbiological quality of irrigation water and incidence of human pathogens on fruits and vegetables, there is limited evidence of the outbreaks associated with irrigation water, due to the difficulty of narrowing down the exact source responsible (Harris et al., 2013).

Wild animals and their activities in the agricultural field have been reported to be the causes of produce contamination (Kwan et al., 2014; Laidler et al., 2013; Weller et al., 2017). Wildlife may defecate on agricultural land resulting in contamination of the growing fresh produce (Holvoet, Sampers, Seynnaeve, & Uyttendaele, 2014). The respiratory systems, skin, hooves, and hair or feathers of animals are the sources of human pathogens, because pathogenic bacteria may survive inside them without causing harm (Ray & Bhunia, 2013). Their intrusion into the agricultural farm may cause the transfer of pathogens from these parts to the edible portion of produce. Moreover, they might damage the leaves or other parts of fresh produce providing entry point for foodborne pathogens into the plant (Orozco et al., 2008).

2.1.2. Post-harvest contamination

Post-harvest contamination can occur at any time after harvest; this includes contamination in the field, field packing, processing, and contamination in retail stores. The main method of

avoiding post-harvest contamination revolves around proper Good Agricultural Practices (GAP´s), Good Hygene Practices (GHP´s) and Good Manufacturing Practices (GMP´s) (Allende et al., 2015). However, many times produce contamination has proven to be sporadic and difficult to control effectively before the product reaches the consumer. FDA and USDA studies on imported produce revealed a prevalence of *Salmonella* and *Shigella* contamination on 50% of cilantro, 7.3% cantaloupe, 3.6% celery, 2.4% parsley, 1.7% lettuce and 1.7% scallions. Domestic produce has proven to have lower prevalence; however, certain commodities have still proven to be an issue with scallions and cantaloupes, presenting the highest risks (Gerba et al., 2014).

On-farm contamination is highly common, due to many agricultural practices that already involve a high produce safety risk (Gerba et al., 2014). Soil amendments such as compost, by its nature, has a high microbial population, and its improper preparation or application can lead to an increase in pathogen levels in the amendments (Barton et al., 2015). Agricultural water is not required to be free of bacteria since water sources such as well water will contain bacteria such as non-pathogenic *E. coli*. However, this also means that the spread of agricultural water may increase the radius of contamination if the source were to be contaminated with pathogenic bacteria (Amoah et al., 2015; Gradl & Worosz, 2017). Animal intrusions are difficult to contain, especially in farms with a large area to monitor (Aceituno et al., 2017; Gerba et al., 2014). These intrusions can contaminate produce and if they are not identified in a specific zone, then this contamination may spread once the whole field is harvested. Good Agricultural Practices, therefore make emphasis on zonal prevention of contamination and proper management of high food safety risks like water and soil amendments.

Contaminations in packing facilities or processing plants despite being in an environment with less risk than in the field produce is still at a high risk of being contaminated with pathogens.

One of the main routes of contamination in these environments is employees, poor personal hygiene or poor management of the produce can increase the risk of contamination (Aceituno et al., 2017). Bacteria that are prevalent in processing facilities have presented a major issue. Pathogens such as *Listeria monocytogenes* are able to form biofilms inside these environments, prevailing due to frequent and efficient cleaning protocols. The biofilms are hotspots for contamination since they continue to spread contamination to the rest of the products that are processed in these facilities (Barbuddhe et al., 2015; Goldfine & Shen, 2007). The main control for reducing these risks are treatments with sanitizers, mostly involving chlorine. The wash treatments consists of three stages; the first being focused on eliminating debris and organic material, the second stage uses a sanitizer in order to reduce cross-contamination and reduce microbial load and the third stage uses non-chlorinated water to rinse the product (Allende et al., 2015; Caleb, Geyer, Mahajan, Singh, & Watkins, 2014).

2.3. Microbial survival and growth in produce

Several studies reported the survival behavior of bacterial cells on produce surfaces. *E. coli* and *Salmonella* survived on parsley in the field for several days (Islam, Morgan, Doyle, & Jiang, 2004). (Stine, Song, Choi, & Gerba, 2005) observed that human pathogens were able to survive for 14 days on cantaloupe, lettuce, and pepper. However, in the plant area above ground, human pathogens are required to adapt to a number of extreme and fluctuating environmental conditions combined with unique physio-chemical characteristics to survive and grow (Berger et al., 2010). For example, *Pseudomonas* spp. protects itself from UV light by producing pigments (Heaton & Jones, 2008). The environmental stress in agricultural fields includes solar radiation, relative

humidity, temperature, availability of nutrients and interaction with other natural microflora (Brandl, 2006; Weller et al., 2017). If the bacterial cells are unable to adapt to environmental stress, their die-off occurs over time. Sunlight of tropical latitudes (Davies, 2003; Nyeleti, Cogan, & Humphrey, 2004; Obiri-Danso, Paul, & Jones, 2001) and concomitant increase in the surface temperature of produce have an inhibitory effect against various microbial pathogens (Tomas-Callejas et al., 2011). The combined effect of greater temperatures and drying conditions could efficiently reduce the microbial load (Van Donsel, Geldreich, & Clarke, 1967). It was observed that sunlight reduced *Salmonella* levels in freshwater sources (Davies, 2003). Lack of nutrients on phyllosphere could be another stress factor for microbial inhibition (Fontaine, Mariotti, & Abbadie, 2003).

FDA Food Safety Modernization Act (FSMA) produce safety rule (PSR) has considered the natural die-off of bacterial cells as a corrective measure to reduce risks associated with agricultural water and biological soil amendments (Gradl & Worosz, 2017). The growers unable to meet microbial water quality criteria could use a die-off rate of 0.5 log per day while calculating the waiting period between the last irrigation and harvest. However, the die-off rate may be influenced by several factors such as surface characteristics of produce and the geographical location, as well as ripeness with tissues softening and nutrients becoming more available (Gradl & Worosz, 2017; Weller et al., 2017).

2.4. Knowledge gaps

Despite antimicrobial packaging being a very wide and investigated field of the food industry, there are still several areas that need further investigations. There are several different and effective antimicrobials; however, these antimicrobials are very specific to certain microorganisms which limits their general use in any product. This becomes an issue because many times a food may be susceptible to different kinds of bacteria or fungi and the antimicrobial applied will only control a small amount of microorganisms. An effective control for both gram-positive and gram-negative bacteria is needed Which could be with an effective combination of antimicrobials or a single antimicrobial.

More investigation is needed concerning different polymers that can be used for packaging. Considering that environmental impacts must be mitigated proper degradable packages should be tested in order to see how viable they may be. In addition, most of the investigations of antimicrobial packaging are oriented towards products that already have several advances in proper packaging, such as dairy and meat products. However most foodborne outbreaks are due to contamination of fruits and vegetables, creating a grey area inside this area of packaging, which must urgently be addressed. Most of these outbreaks are due to L. monocytogenes, therefore proper methods of controlling this microorganism in produce are required.

3. EFFECT OF STORAGE TEMPERATURE ON THE SURVIVAL OR GROWTH OF *Listeria monocytogenes* ON WHOLE AND FRESH-CUT PRODUCE

3.1. Introduction

In 2018, the total production of produce in the United States accounted for 753 million cwt. The USDA calculated this production to represent a value of $12.9 billion in 2018 alone (USDA, 2019b). Roughly, about half of this production is used for fresh-market consumption, with 358.9 million cwt of the total production going to the fresh-market in 2018 (USDA, 2019a). Fresh-produce due to its origin of production is highly susceptible to contamination by pathogenic bacteria (Aceituno et al., 2017). One bacteria of particular importance to this type of products is *Listeria monocytogenes*, since this pathogen is a ubiquitous bacterium as it can be naturally found in environments such as water and soil. Once *L. monocytogenes* enters the processing facilities it can survive for long periods due to favorable humidity and temperature (Dalmasso et al., 2015).

Fresh produce generally does not undergo harsh processing conditions such as pasteurization or sterilization, due to the effect that these conditions have on the quality of the product (Olaimat & Holley, 2012). Sanitizer treatments are most commonly used in fresh produce processing, because of its less invasive nature. However, they are not as efficient as heat treatments (Keskinen et al., 2009). Therefore, fresh produce processors must focus their efforts mainly in prevention of contamination, instead of controlling or reducing microbial growth (Kumar et al., 2015).

Temperature is a key factor in either slowing down or increasing the growth of bacteria on fresh produce surfaces (M. D. Danyluk et al., 2012). The major issue with using temperature as a control is that bacteria are not reduced by low temperature; they are just forced into a latent state. Even more troublesome, pathogens such as *Listeria monocytogenes* are able to grow at refrigeration temperatures, this coupled with the ripening process due to ethylene production and also tissue damage caused by low temperatures, makes contaminations easier to occur (Dodd, Nwaiwu, & Rees, 2013; Goldfine & Shen, 2007). Some fruits and vegetables by themselves provide certain barriers for microbial growth, such as acidic pH´s, but even these many times have proven to not be enough to avoid microbial growth (Berger et al., 2010).

The growth of pathogens like *Listeria monocytogenes* can at times be difficult to predict in specific surfaces (East et al., 2016). The produce safety rule suggests that in general bacteria will begin to die-off as time goes by, but this is not always the case in specific surfaces. Considering these knowledge gaps the objectives of this study were as follows:

- Examine the growth or survival of *Listeria monocytogenes* on the surface of fresh-cut and vegetables during different storage temperatures.
- Examine the growth or survival of *Listeria monocytogenes* on whole cantaloupes and green bell peppers during different storage temperatures.

3.2. Materials and Methods

3.2.1. Sample preparation

Mature fruit (Sol Group cantaloupes , pears, honey seeded variety watermelons, papayas var Maradol and pineapples var Piña Miel) and leafy green vegetables (Lettuce, kale, cauliflower, broccoli, and green bell peppers) were purchased from local wholesale markets and stored in the refrigerator at 4°C for future use.

The rinds of cantaloupes and green bell peppers were prepared under aseptic conditions by initially tracing a circle of 20 cm^2 using a sterilized VWR plastic bottle cap. These circles were cut out using a disinfected knife, removing the internal mesocarp in order to only leave the external layers as part of the sample. Rinds were then placed on previously labeled Petri dishes without covers and transferred into a biosafety hood. The samples were then inoculated with either 4 Log CFU/mL or 6 Log CFU/mL of inoculum. After one hour of being dried inside the biosafety hood, the samples were stored in an incubator at 24°C.

Fresh-cut samples were prepared by cutting fruits or vegetables into cubes totalling 25g with a disinfected knife. In the case of fruit samples, only the mesocarp was used. These samples were then placed in previously labeled uncovered Petri dishes and transferred into the biosafety hood. Each replicate was prepared by approximately, two cantaloupes, three papayas, two pineapples, six pears, and one watermelon for fruit samples. For vegetable samples each replicate was prepared from approximately, six green bell peppers, two cauliflower heads, two broccoli heads, one lettuce head and a bag of 500g of kale. The pH of the samples was taken during the first and last day of analysis with a VWR H30PCO Multi-Parameter Handheld Meter, by grinding

samples in order to release juices and then measure the pH.. All samples were inoculated with 4 Log CFU/mL. After one hour of being dried inside the biosafety hood, samples were transferred to incubators at either 4°C or 13°C.

3.2.2. Inoculum preparation

Four strains of *Listeria monocytogenes* (LCDC 81-861 (4b), V7 (1/2a), 101 M (4b), and Scott A (4b)) were obtained from the University of Florida with the collaboration of Dr. Michelle Danyluk.

All cultures were kept in the freezer at -20°C and then routinely activated as follows: the frozen cultures were thawed, vortexed (Fischer Scientific vortex), and 0.1mL was transferred into 10ml test tubes of Tryptic Soy Broth (Criterion) containing 0.6% yeast extract (TSBYE) obtained from VWR company. The TSBYE test tubes were supplemented with Nalidixic Acid at a concentration of 50 µg/mL and were later incubated at 37ºC for 24 hours. Nalidixic Acid was used in order to make the media more selective and mainly allow the growth of *L. monocytogenes*. After incubation, 1mL was transferred to a fresh 10mL test tube of TSBYE and incubated at 37ºC for 24 hours, and then 0.1mL was transferred to another fresh 10mL test tube of TSBYE and incubated at 37ºC for 24 hours. Cells were then harvested after a total of 72 hours of incubation by centrifugation (Labnet Spectrafuge 6C Centrifuge) at 6500 rpm for 5 minutes using Falcon tubes at room temperature. After centrifugation, the supernatant was discarded, and 10 mL of Phosphate Buffer Saline 1x (PBS) was added followed by centrifugation as described above. Supernatants were discarded, and pellets were resuspended in 10 mL of PBS 1x. The cocktail of *L. monocytogenes*

was prepared at a cell density of 8 Log CFU/mL by mixing the strains suspension in a 50mL plastic tube. The cocktail was then serially diluted to 4 Log CFU/mL or 6 Log CFU/mL as needed.

3.2.3. Inoculation and storage conditions of samples

Fresh-cut and rind samples of cantaloupes and green bell peppers were spot inoculated with 0.5 ml of either low (4 Log CFU/ml) or high (6 Log CFU/ml) inoculum. Fresh-cut cantaloupes, fresh-cut green bell peppers and green bell pepper rind samples were kept at 4°C for storage while cantaloupe rinds were stored at 24°C. Fresh-cut fruit and vegetables under different storage conditions were spot inoculated with 0.5 ml of low (4 Log CFU/ml) inoculum. Samples were left to dry inside the biosafety laminar flow hood for one hour. Afterward samples were transferred to either 4°C, 13°C or 24°C storage conditions. Fresh-cut samples were stored at 4°C or 13°C during 6 days, green bell pepper rind samples were stored at 4°C or 13°C during 14 days, and cantaloupe rinds were stored at 24°C for 8 days. Storage temperatures were decided considering at what temperatures the samples are usually hold by consumers, and the length of analysis was decided by doubling the regular shelf-life of each product.

3.2.4. Sample Analysis

Rind samples were removed from incubation and placed inside a biosafety hood, using an autoclaved peeler the skin of the rind was peeled off and placed in a Fisher brand centrifuge tube. The sample was then weighed, deducting the weight of the centrifuge tube, and this information

was recorded. Afterward 20mL of DE Neutralizing Broth was added to the centrifuge tube and with a disinfected scissor, the sample was cut into smaller pieces. A vertical Fisher Scientific 150 Handheld Homogenizer was used to homogenize the sample. Later 1ml of the sample was extracted, and it was diluted accordingly in 9mL test tubes of 0.1% peptone water. Samples were then plated on Oxford Listeria Agar supplemented with Nalidixic Acid. These plates were then incubated at 37°C for 24 hours.

Fresh-cut samples were removed from incubation and placed inside a biosafety hood in which the pH was determined for samples corresponding to the first and last day of analysis, using a VWR H30PCO Multi-Parameter Handheld Meter. The sample was then transferred to Nasco Whirl-Pack Filter Bags, and the weight was recorded. Then 100ml of DE Neutralizing Broth was added to the whirl-pack filter bag, and the bag was stomached for 5 minutes using Interscience Bag Mixer SW. The sample was then diluted accordingly in 9mL test tubes of 0.1% Peptone Water, and 0.1mL was plated on Oxford Listeria Agar Plates supplemented with Nalidixic Acid. The plates were then incubated at 37°C for 24 hours in a Sanyo MCO-20AIC CO_2 Incubator. Yeast and Mold, and Aerobic Plate Count 3M Petri films were plated and incubated on the first and last day of analysis for fresh-cut samples, according to the manufacturer´s specifications.

3.2.5. Microbial Analysis

After 24 hours of incubation at 37°C, Oxford Listeria Agar plates were removed from the Sanyo MCO-20AIC CO_2 Incubator, and the colony-forming units were counted. The microbial

population on rind samples was calculated according to Log CFU/cm^2 and fresh-cut samples were calculated according to Log CFU/g. Rind samples that were suspected to be below the detectable limit for agar plates were placed in bottles of 100mL Tryptic Soy Broth with Yeast Extract and incubated at 37°C for 24 hours, in a Sanyo MCO-20AIC CO_2 Incubator. If the plates presented no counts, then these incubated samples were plated on new Oxford Listeria Agar plates supplemented with Nalidixic Acid and incubated at 37°C for 24 hours. This determined absence or presence of *L. monocytogenes*. Fresh-cut samples that were suspected of being below the detectable limit were plated by inoculating from a 10^0 dilution; 300 µL, 300 µL, and 400 µL in three separate Oxford Listeria Agar Plates supplemented with Nalidixic Acid and incubated at 37°C for 24 hours. This was done in order to complete 1 mL of inoculum, which is more representative than 0.1 mL. Yeast and Mold Petri films were counted after three days of incubation, afterward which the Petri films were counted three times after 24 hours each time, as per the instructions of the manufacturer. Aerobic Plate Count Petri films were counted twice, after 24 and 48 hours, as per the instructions of the manufacturer.

3.2.6. Statistical Analysis

Dataset Description:

- Produce: Ten different types of produce where analyzed during the study. These included five fresh-cut fruit samples which were; pear, papaya, pineapple, watermelon and cantaloupe. The rest of the samples were fresh-cut vegetables, which were; broccoli, cauliflower lettuce, kale and green bell peppers. Rind samples for green bell peppers and cantaloupe were also analyzed. These samples were spot inoculated with *L. monocytogenes*.

- Storage Temperature: This variable consisted of the temperature the samples were stored at during the analysis; this could be either 4°C, 13°C or 24°C. Samples were stored at refrigeration incubators in order to maintain constant temperatures.
- Sampling Times: The inoculated produce was periodically sampled in order to determine if the storage temperatures would have an effect on the survival or growth of *L. monocytogenes*. These sampling times were at 0, 1, 2, 4, and 6 days for fresh-cut samples. Sampling days for green bell pepper rinds were 0, 1, 2, 6, 10, and 14 days. Sampling days for cantaloupe rinds were 0, 1, 2, 4, 6, and 8 days.
- Microbial Counts: This variable was the population of *L. monocytogenes* present on the surface of the fresh-cut produce at a certain storage temperature and sampling time. All the counts were reported as Log CFU/ g.

Table 3.1 Sample of Dataset for Fresh-cut Produce

Produce	Storage Temperature	Sampling Times	Microbial Counts
Pear	4°C	0	3.24
Pear	4°C	1	3.37
Pear	4°C	2	3.34
Pear	4°C	4	3.58
Pear	4°C	6	3.51
Pear	13°C	0	3.38
Pear	13°C	1	3.50
Pear	13°C	2	4.36
Pear	13°C	4	4.88
Pear	13°C	6	5.17

The experimental design for this study consisted of a Completely Randomized Design. The data were analyzed using Statistical Analysis Software 9.4, with a post ANOVA, Tukey mean separation, LS Means and a probability <0.05.

Statistical Model:

- ANOVA: Analysis of Variance is a statistical test that evaluates if there is a significant difference between the means of multiple groups. This test is combined with a post ANOVA test described below to better analyze the difference between these groups (Freund, Wilson, & Mohr, 2010).
- LS Means: Least Square Means analysis is a statistical test that allows us to determine if there are significant differences in between the variables present in the study. This test also allows to determine if there are any significant interactions between the variables. If any of the interactions was determined to be significant, then a Least Square Means for Effect table would allow us to determine which means were significantly different from each other and which ones were the same (Freund et al., 2010).
- General Linear Model: Linear modelling is a statistical tool to determine if there is a significant impact on a dependent variable by one or several independent variables. This is a form of predictive modelling that serves to predict future results based on the actual values of a dataset (Freund et al., 2010).

3.4. Results and discussion

3.4.1. Survival or growth of *Listeria monocytogenes* in fresh-cut fruits

The growth or survival of *L. monocytogenes* on five different fresh-cut fruit samples during storage is shown in Figures 4.1 and 4.2. The bacterial growth patterns at the storage temperatures of 4ºC and 13ºC were different. At 4º C, a significant reduction in the level of *L. monocytogenes* was observed on fresh cut-pineapple samples. While on other fresh-cut fruit samples, fresh-cut pears, papaya, pineapple, and watermelon, there were not significant changes in bacterial population up to day 6 (Figure 4.1). Although there was a reduction in the bacterial population (by 1.14 Log CFU/g) in pineapples after 6 days of storage, the level of reduction was not statistically significant (Table 4.1). The role of refrigeration temperature in controlling the growth of bacteria in fresh produce has been demonstrated by several studies. Abadias and colleagues (2015) encountered different results. They observed increases in *L. monocytogenes* levels by up to 2.05 Log CFU/g in pears stored at 10°C in 8 days. This discrepancy between our results and their results may be due to the differences in pear type used (Abadias, Alegre, Colás-Medá, Usall, & Viñas, 2015). Bai and colleagues (2015) found that that the storage conditions of 4-5°C were enough to stop the growth of *L. monocytogenes* in papaya surfaces (Bai et al., 2015). A study carried out by Huang and colleagues (2019) highlights the inability of this pathogen to grow at constant refrigeration temperatures of 4-5°C. Huang and colleagues encountered slow populations increases by up to 1.60 Log CFU/g on the surface of fresh-cut cantaloupe and watermelon samples (J. Huang et al., 2019). The survival of *L. monocytogenes* on cantaloupe surface is in accordance with Danyluk and colleagues´s study (2014) in which growth models developed for a storage temperature of 4°C and 6 days of analysis for cantaloupes predicted an approximate increase of up to 1 Log CFU/g at 4°C conditions (M. Danyluk, Friedrich, & Schaffner, 2014)

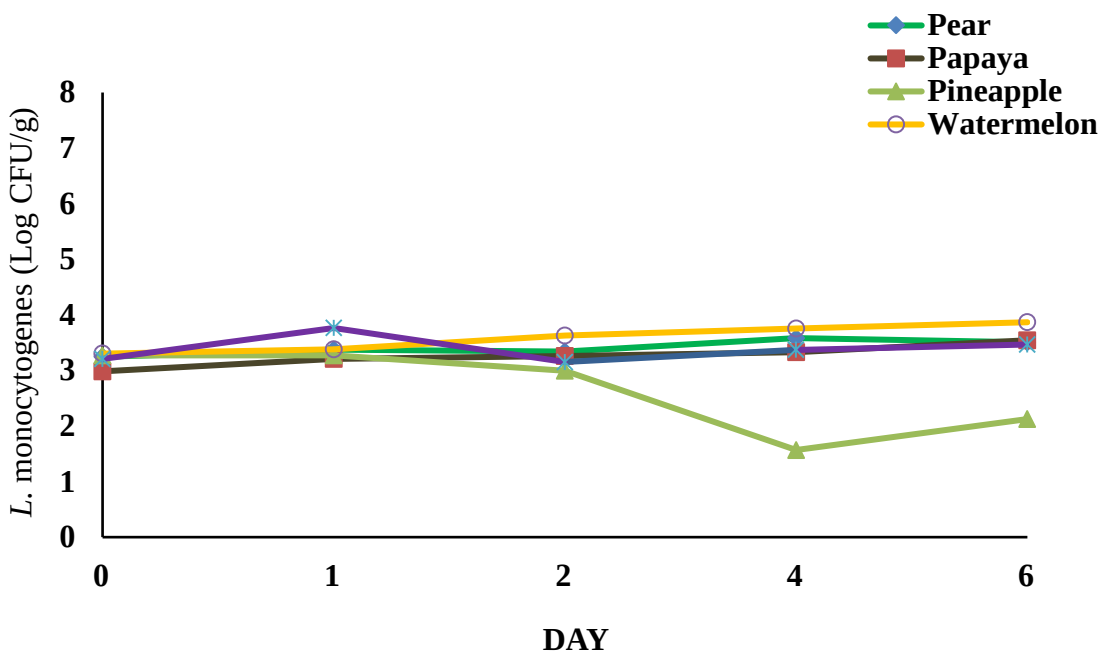

Figure 4.1. Survival of Listeria monocytogenes inoculated on fresh-cut fruit samples stored at 4°C.

Table 4.1. Pineapple LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.6979	0.1186	0.0505	0.0501	0.8017	0.8032	0.5275	0.0444	0.1120
2	0.6979		0.1928	0.0786	0.0779	0.8888	0.8873	0.7978	0.0684	0.1818
3	0.1186	0.1928		0.4766	0.4719	0.1619	0.1615	0.2665	0.4130	0.9626
4	0.0505	0.0786	0.4766		0.9932	0.0669	0.0668	0.1066	0.9042	0.5033
5	0.0501	0.0779	0.4719	0.9932		0.0663	0.0662	0.1056	0.9109	0.4984
6	0.8017	0.8888	0.1619	0.0669	0.0663		0.9985	0.6942	0.0584	0.1527
7	0.8032	0.8873	0.1615	0.0668	0.0662	0.9985		0.6928	0.0583	0.1523
8	0.5275	0.7978	0.2665	0.1066	0.1056	0.6942	0.6928		0.0923	0.2512

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
9	0.0444	0.0684	0.4130	0.9042	0.9109	0.0584	0.0583	0.0923		0.4369
10	0.1120	0.1818	0.9626	0.5033	0.4984	0.1527	0.1523	0.2512	0.4369	

Table 4.2. Pear LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.5864	0.0070	0.0015	0.0007	0.5187	0.9734	0.8488	0.3573	0.5515
2	0.5864		0.0107	0.0020	0.0010	0.2642	0.5650	0.4715	0.6769	0.9561
3	0.0070	0.0107		0.0549	0.0137	0.0044	0.0068	0.0061	0.0153	0.0112
4	0.0015	0.0020	0.0549		0.2057	0.0011	0.0014	0.0013	0.0025	0.0020
5	0.0007	0.0010	0.0137	0.2057		0.0006	0.0007	0.0007	0.0012	0.0010
6	0.5187	0.2642	0.0044	0.0011	0.0006		0.5388	0.6411	0.1557	0.2465
7	0.9734	0.5650	0.0068	0.0014	0.0007	0.5388		0.8749	0.3429	0.5310
8	0.8488	0.4715	0.0061	0.0013	0.0007	0.6411	0.8749		0.2818	0.4419
9	0.3573	0.6769	0.0153	0.0025	0.0012	0.1557	0.3429	0.2818		0.7164
10	0.5515	0.9561	0.0112	0.0020	0.0010	0.2465	0.5310	0.4419	0.7164	

At 13^{o}C, significant growth of *L. monocytogenes* was observed in all the fresh-cut produce except on pineapples (Figure 4.2 and Table 4.1). On pears and papaya, there were no significant changes in the bacterial population until the second day (Tables 4.2 and 4.3). However, in subsequent days, the population increased significantly compared to the initial count. The level increased by 1.79 Log CFU/g and 3.8 Log CFU/g in fresh-cut pears and papaya, respectively.

The *Listeria* population showed significant growth in watermelons and cantaloupes from the first day of the storage, with a total increase in population by 3.45 Log and 4.24 Log CFU/g, respectively (Tables 4.4 and 4.5). Among the tested fresh-cut produce, the highest growth was observed in cantaloupe, followed by papaya, watermelon, and pears. In pineapples, there was a reduction in the population over time with a total reduction by 1.80 Log CFU/g after 6 days of storage. However, the reduction was not statistically significant, compared to the initial count. The inability of *Listeria* to grow in pineapple could be attributed to the antimicrobials present in the fruits. The results obtained for fresh-cut pear samples are similar to a study carried out in 2017, in which inoculated pear samples stored at 10°C presented an increase of 2.05 Log CFU/g after 7 days of storage (Abadias et al., 2017). Abadias and colleagues (2017) also determined that cantaloupes provided an appropriate environment for the growth of *L. monocytogenes* with an increase in the population by 3.88 Log CFU/g (Abadias et al., 2017). One of the most likely reasons is the highly acidic pH in pineapple; however, recent studies indicate the enzyme bromelain may have antimicrobial properties (Bai et al., 2015; Dewi, Hemiawati, & Loon, 2018). Erfan and colleagues in (2018) used isolated bromelain extracts and were able to inhibit the growth of *Enterococcus faecalis*, a gram-positive bacteria (Erfan, Lillany, Sadono, Sari, & Sudiono, 2018). However, further studies may be needed in order to determine the exact conditions that make pineapple an inadequate growth medium for *L. monocytogenes*

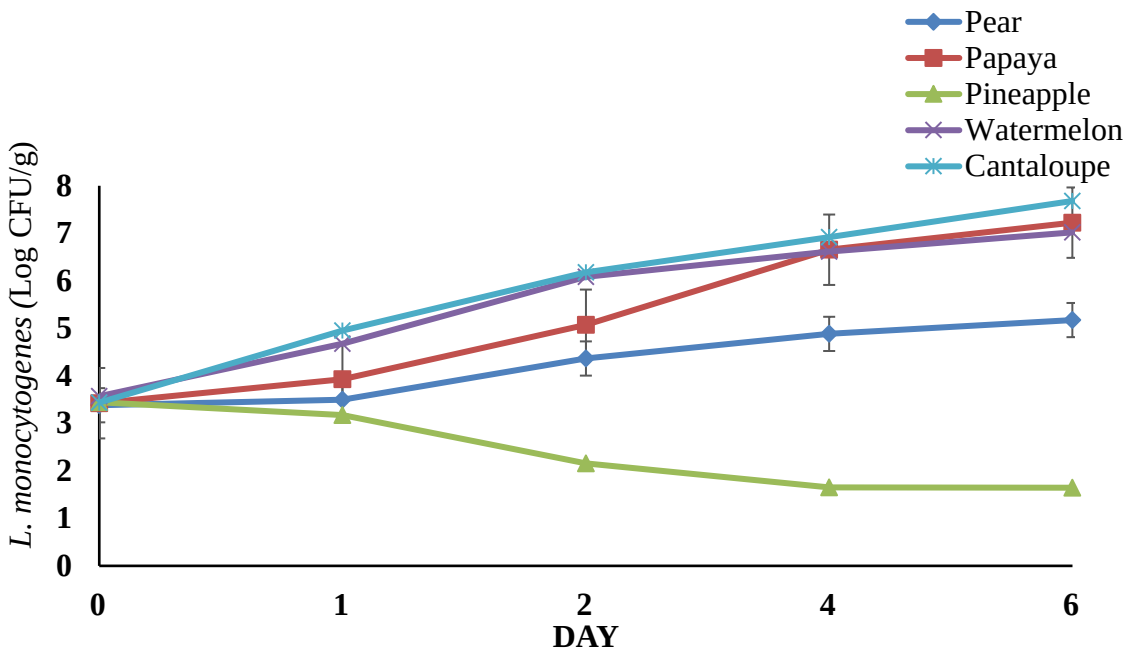

Figure 4.2. Survival of *Listeria monocytogenes* inoculated on fresh-cut fruit samples stored at 13°C.

Table 4.3. Papaya LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.0743	0.0014	0.0001	<.0001	0.1031	0.3603	0.4777	0.6771	0.6008
2	0.0743		0.0054	0.0002	<.0001	0.0108	0.0265	0.0334	0.0464	0.1406
3	0.0014	0.0054		0.0017	0.0005	0.0006	0.0009	0.0010	0.0011	0.0019
4	0.0001	0.0002	0.0017		0.0528	<.0001	<.0001	<.0001	<.0001	0.0001
5	<.0001	<.0001	0.0005	0.0528		<.0001	<.0001	<.0001	<.0001	<.0001
6	0.1031	0.0108	0.0006	<.0001	<.0001		0.3439	0.2566	0.1730	0.0557
7	0.3603	0.0265	0.0009	<.0001	<.0001	0.3439		0.8150	0.5906	0.1849
8	0.4777	0.0334	0.0010	<.0001	<.0001	0.2566	0.8150		0.7551	0.2484
9	0.6771	0.0464	0.0011	<.0001	<.0001	0.1730	0.5906	0.7551		0.3672

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
10	0.6008	0.1406	0.0019	0.0001	<.0001	0.0557	0.1849	0.2484	0.3672	

Table 4.4. Watermelon LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.0079	0.0004	0.0002	0.0001	0.2976	0.4384	0.8194	0.4545	0.2579
2	0.0079		0.0033	0.0010	0.0005	0.0036	0.0044	0.0094	0.0148	0.0226
3	0.0004	0.0033		0.0768	0.0140	0.0002	0.0003	0.0004	0.0005	0.0006
4	0.0002	0.0010	0.0768		0.1461	0.0001	0.0001	0.0002	0.0002	0.0003
5	0.0001	0.0005	0.0140	0.1461		<.0001	<.0001	0.0001	0.0001	0.0001
6	0.2976	0.0036	0.0002	0.0001	<.0001		0.7532	0.2232	0.1130	0.0657
7	0.4384	0.0044	0.0003	0.0001	<.0001	0.7532		0.3317	0.1669	0.0950
8	0.8194	0.0094	0.0004	0.0002	0.0001	0.2232	0.3317		0.5908	0.3432
9	0.4545	0.0148	0.0005	0.0002	0.0001	0.1130	0.1669	0.5908		0.6493
10	0.2579	0.0226	0.0006	0.0003	0.0001	0.0657	0.0950	0.3432	0.6493	

Table 4.5. Cantaloupe LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.0046	0.0005	0.0002	<.0001	0.4325	0.2851	0.3420	0.8133	0.9125
2	0.0046		0.0099	0.0017	0.0005	0.0027	0.0109	0.0024	0.0039	0.0050
3	0.0005	0.0099		0.0479	0.0047	0.0004	0.0008	0.0003	0.0004	0.0005

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
4	0.0002	0.0017	0.0479		0.0454	0.0001	0.0003	0.0001	0.0002	0.0002
5	<.0001	0.0005	0.0047	0.0454		<.0001	0.0001	<.0001	<.0001	<.0001
6	0.4325	0.0027	0.0004	0.0001	<.0001		0.1031	0.8473	0.5691	0.3788
7	0.2851	0.0109	0.0008	0.0003	0.0001	0.1031		0.0820	0.2117	0.3270
8	0.3420	0.0024	0.0003	0.0001	<.0001	0.8473	0.0820		0.4557	0.2984
9	0.8133	0.0039	0.0004	0.0002	<.0001	0.5691	0.2117	0.4557		0.7307
10	0.9125	0.0050	0.0005	0.0002	<.0001	0.3788	0.3270	0.2984	0.7307	

3.4.2. Microbial survival or growth of *Listeria monocytogenes* in fresh-cut vegetable samples.

The growth or survival of *L. monocytogenes* on five different vegetable samples during storage is shown in Figures 4.3 and 4.4. There was a decrease in *L. monocytogenes* populations with time on broccoli, cauliflower, lettuce, and kale under the storage temperature of 4°C up to 48 h. Cauliflower and broccoli had significant reductions ($P < 0.05$) in the bacterial populations, by 1.03 Log CFU/g and 0.78 Log CFU/g, respectively, at 4 days of storage (Tables 4.6 and 4.7). Although there was a decrease in the bacterial population on lettuce (by 0.42 Log CFU/g) and kale (0.73 Log CFU/g), the level of reduction was not significantly different (Tables 4.8 and 4.9). While on green pepper, the bacterial population remains stable up to 48 h (Table 4.10).

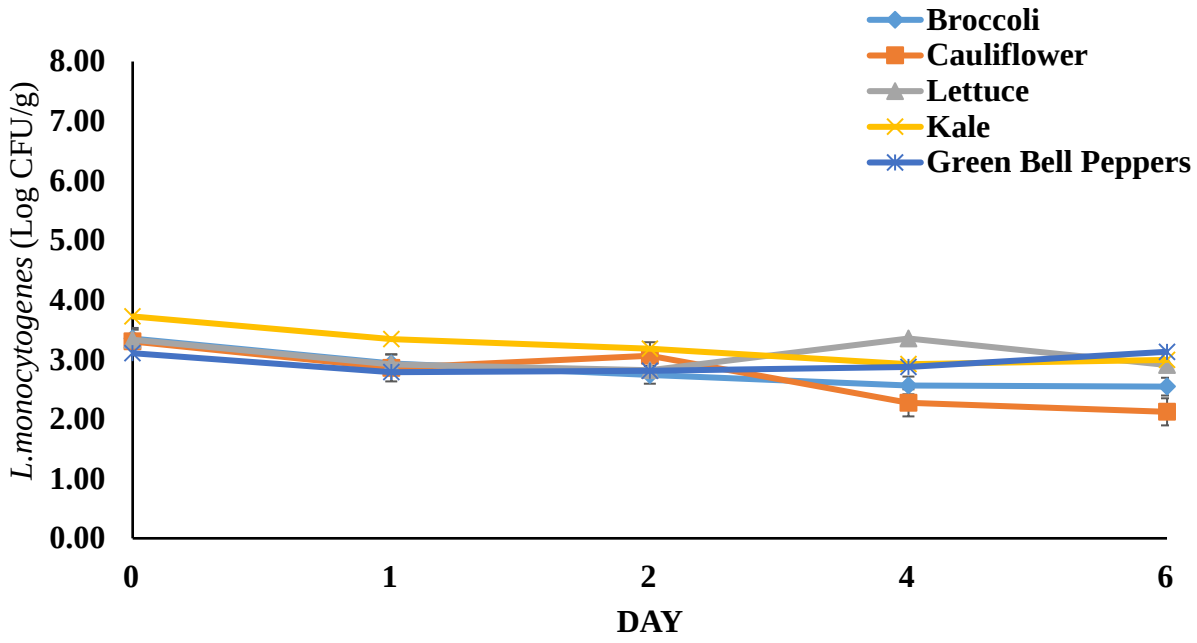

Figure 4.3. Survival of *L. monocytogenes* inoculated on fresh-cut vegetable samples stored at 4°C.

Table 4.6. Broccoli LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.6283	0.2214	0.1190	0.1016	0.6661	0.1014	0.0294	0.0115	0.0104
2	0.6283		0.1201	0.0666	0.0575	0.9561	0.0574	0.0184	0.0078	0.0071
3	0.2214	0.1201		0.6231	0.5392	0.1284	0.5383	0.1347	0.0408	0.0362
4	0.1190	0.0666	0.6231		0.8962	0.0709	0.8950	0.2515	0.0708	0.0620
5	0.1016	0.0575	0.5392	0.8962		0.0611	0.9988	0.2962	0.0823	0.0719
6	0.6661	0.9561	0.1284	0.0709	0.0611		0.0610	0.0194	0.0081	0.0074
7	0.1014	0.0574	0.5383	0.8950	0.9988	0.0610		0.2967	0.0824	0.0721
8	0.0294	0.0184	0.1347	0.2515	0.2962	0.0194	0.2967		0.3306	0.2861

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
9	0.0115	0.0078	0.0408	0.0708	0.0823	0.0081	0.0824	0.3306		0.9077
10	0.0104	0.0071	0.0362	0.0620	0.0719	0.0074	0.0721	0.2861	0.9077	

Table 4.7. Cauliflower LS Means Outputs

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.3287	0.0558	0.7715	0.1358	0.7307	0.0589	0.1878	0.0050	0.0031
2	0.3287		0.0194	0.2280	0.4936	0.2128	0.0203	0.0542	0.0026	0.0017
3	0.0558	0.0194		0.0777	0.0105	0.0828	0.9610	0.3393	0.0428	0.0209
4	0.7715	0.2280	0.0777		0.0953	0.9562	0.0822	0.2710	0.0061	0.0038
5	0.1358	0.4936	0.0105	0.0953		0.0893	0.0110	0.0260	0.0017	0.0012
6	0.7307	0.2128	0.0828	0.9562	0.0893		0.0877	0.2903	0.0064	0.0039
7	0.0589	0.0203	0.9610	0.0822	0.0110	0.0877		0.3604	0.0407	0.0200
8	0.1878	0.0542	0.3393	0.2710	0.0260	0.2903	0.3604		0.0159	0.0088
9	0.0050	0.0026	0.0428	0.0061	0.0017	0.0064	0.0407	0.0159		0.4866
10	0.0031	0.0017	0.0209	0.0038	0.0012	0.0039	0.0200	0.0088	0.4866	

At 13°C, the tested vegetables generated mixed results with some samples allowing the pathogens to survive and others supported their growth. Broccoli samples had no significant changes in the bacterial population up to 48 h under the storage temperature of 4°C. The initial population on the broccoli was 3.28 Log CFU/g which decreased to 2.94 Log CFU/g (Figure 4.4). Likewise, cauliflower and kale had no significant changes in the bacterial population (Tables 4.7

and 4.9). While on green bell peppers the *Listeria* count increased significantly (1.02 Log CFU/g) after the second day of storage. Similarly, on lettuce samples, the population increased significantly by 0.82 Log CFU/g after 6 days of storage (Tables 4.8 and 4.10). The results indicated that bell pepper and lettuce provided a better Listeria growth condition compared to other produce under both tested temperatures, 4ºC and 13ºC.

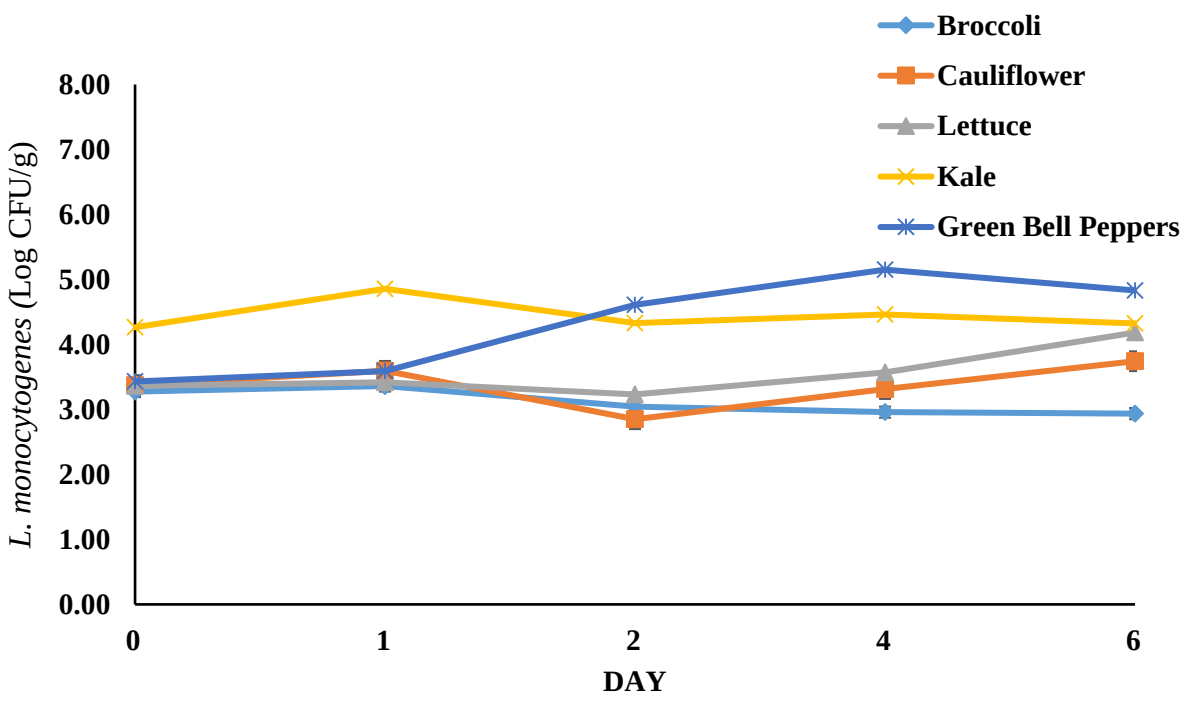

Figure 4.4. Survival of *Listeria monocytogenes* inoculated on fresh-cut vegetable samples stored at 13°C.

Table 4.8. Lettuce LS Means Outputs

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.8616	0.6696	0.5055	0.0450	0.9123	0.1951	0.1304	0.9737	0.1856
2	0.8616		0.5537	0.6149	0.0544	0.7769	0.1568	0.1054	0.8360	0.1492
3	0.6696	0.5537		0.2997	0.0289	0.7493	0.3352	0.2234	0.6930	0.3190
4	0.5055	0.6149	0.2997		0.0981	0.4442	0.0843	0.0582	0.4865	0.0805
5	0.0450	0.0544	0.0289	0.0981		0.0401	0.0114	0.0088	0.0435	0.0110
6	0.9123	0.7769	0.7493	0.4442	0.0401		0.2241	0.1494	0.9384	0.2131
7	0.1951	0.1568	0.3352	0.0843	0.0114	0.2241		0.7474	0.2034	0.9681
8	0.1304	0.1054	0.2234	0.0582	0.0088	0.1494	0.7474		0.1358	0.7774
9	0.9737	0.8360	0.6930	0.4865	0.0435	0.9384	0.2034	0.1358		0.1934
10	0.1856	0.1492	0.3190	0.0805	0.0110	0.2131	0.9681	0.7774	0.1934	

Table 4.9. Kale LS Means Outputs

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.1187	0.8411	0.5464	0.8464	0.1448	0.0371	0.0224	0.0109	0.0132
2	0.1187		0.1519	0.2564	0.1506	0.0193	0.0072	0.0050	0.0029	0.0034
3	0.8411	0.1519		0.6798	0.9945	0.1132	0.0303	0.0186	0.0093	0.0111
4	0.5464	0.2564	0.6798		0.6750	0.0692	0.0203	0.0129	0.0068	0.0080
5	0.8464	0.1506	0.9945	0.6750		0.1141	0.0305	0.0187	0.0094	0.0112
6	0.1448	0.0193	0.1132	0.0692	0.1141		0.2744	0.1445	0.0553	0.0713
7	0.0371	0.0072	0.0303	0.0203	0.0305	0.2744		0.6150	0.2305	0.3057

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
8	0.0224	0.0050	0.0186	0.0129	0.0187	0.1445	0.6150		0.4340	0.5635
9	0.0109	0.0029	0.0093	0.0068	0.0094	0.0553	0.2305	0.4340		0.8223
10	0.0132	0.0034	0.0111	0.0080	0.0112	0.0713	0.3057	0.5635	0.8223	

Table 4.10. Green bell pepper LS Means Output

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT										
i/j	1	2	3	4	5	6	7	8	9	10
1		0.3664	0.0016	0.0004	0.0008	0.1024	0.0142	0.0162	0.0229	0.1245
2	0.3664		0.0027	0.0005	0.0013	0.0352	0.0066	0.0074	0.0100	0.0417
3	0.0016	0.0027		0.0249	0.2236	0.0006	0.0003	0.0003	0.0004	0.0007
4	0.0004	0.0005	0.0249		0.1083	0.0002	0.0001	0.0001	0.0001	0.0002
5	0.0008	0.0013	0.2236	0.1083		0.0004	0.0002	0.0002	0.0002	0.0004
6	0.1024	0.0352	0.0006	0.0002	0.0004		0.1106	0.1327	0.2128	0.8719
7	0.0142	0.0066	0.0003	0.0001	0.0002	0.1106		0.8815	0.6040	0.0911
8	0.0162	0.0074	0.0003	0.0001	0.0002	0.1327	0.8815		0.7073	0.1090
9	0.0229	0.0100	0.0004	0.0001	0.0002	0.2128	0.6040	0.7073		0.1738
10	0.1245	0.0417	0.0007	0.0002	0.0004	0.8719	0.0911	0.1090	0.1738	

Our results concurred with the findings reported by Tiang *et al.,* 2012 (Tian et al., 2012). They observed no significant changes in *L. monocytogenes* population on romaine lettuce and iceberg lettuce at 4^{o}C up day 12. Chhetri et al., 2019 demonstrated that there were no significant changes in *L. monocytogenes* population on spinach surfaces under refrigerated temperature (4^{o}C)

(Chhetri, Janes, King, Doerrler, & Adhikari, 2019). However, at 15ºC the population significantly increased after day 2, indicating the role of temperature on the growth of *L. monocytogenes* on produce matrices (Tian et al., 2012). Berrang et al., 1989 observed that asparagus and broccoli supported the growth of *L. monocytogenes* at 15ºC (Berrang, Brackett, & Beuchat, 1989). Martinez and colleagues (2015) reported similar results for broccoli and cauliflower with stable *L. monocytogenes* counts up to 8 Log CFU/mL under the storage temperature of 5 or 10°C (Martínez, Nieves, Pina-Pérez, & Sanz-Puig, 2015). Several studies determined that lettuce is not an adequate environment for the growth of *L. monocytogenes* (Guo, 2017; J. Huang et al., 2019). Fletcher and colleagues (2018) highlighted that in order for the bacteria to grow on a surface such as lettuce it must be able to colonize its surface and then start to reproduce. *L. monocytogenes* is able to attach to produce surfaces; however, it fails to colonize on waxy surfaces (Fletcher et al., 2018). Lokerse and colleagues (2016) determined increases in the population from 1.20 to 1.50 Log CFU/g at 7°C in kale samples, evidencing a lack of proper attachment and adequate growth at this temperature (Lokerse, Maslowska-Corker, Wardt, & Wijtzes, 2016). The ability of *L. monocytogenes* in growing on the surface of lettuce under these storage conditions is in part due to the increase in temperature, and due to possible injuries on the surface of lettuce samples. These injuries allow *L. monocytogenes* to become internalized in the sample and colonize samples rapidly (Fletcher et al., 2018). By cutting the green bell peppers, the peel that serves as a natural barrier causes injuries that serve as contamination points for bacteria (Bortolossi et al., 2016). Growth of *L. monocytogenes* in our study may be attributed to the cut surface of the bell pepper, which provided a favorable condition for the bacteria at 15ºC. Our results indicated that storage temperature and produce type are the factors that influence the growth of *L. monocytoge*

3.4.3 Survival or growth of *Listeria monocytogenes* on cantaloupe and green bell pepper rinds.

The changes in *L. monocytogenes* population on cantaloupe rinds and bell pepper rinds under the storage temperature of 24 ^{o} C is shown in Figure 4.5 and 4.6. We observed a similar survival pattern between low and high inoculum samples over time. For cantaloupe rinds, the initial *L. monocytogenes* count on low inoculum samples was 2.23 Log CFU/cm^2, which increased significantly ($P<0.05$) to 6.39 Log CFU/g on day 4. However, the population started to decline in the subsequent days. The bacterial population decreased significantly ($P<0.05$) to 2.77 Log CFU/cm^2 on day 8 (Table 4.11). We observed a similar survival trend on high inoculum samples up to day 6. The initial count was 4.46 Log CFU/cm^2, which peaked to 7.16 Log CFU/cm^2 on day 6. However, the bacterial level decreased significantly in the following day (day 6) to 5.13 Log CFU/cm^2.

Cantaloupe Rind (Low)=0.04621*DAYS + 4.32826

Cantaloupe Rind (High)=0.18142*DAYS + 4.73753

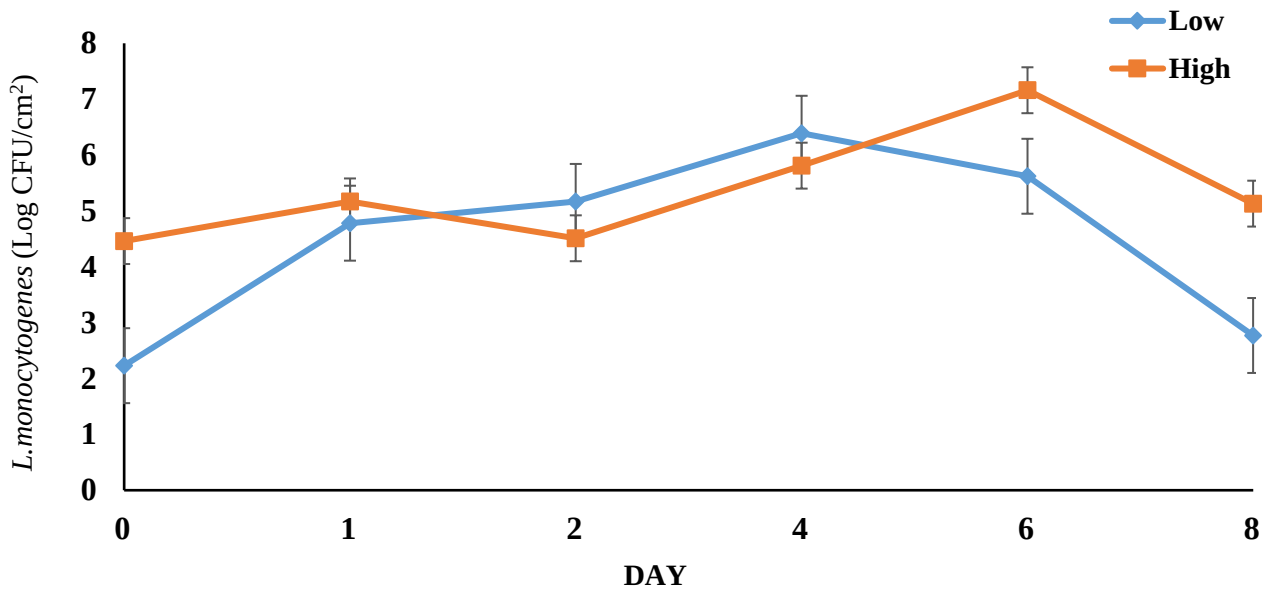

Figure 4.5. Survival of *L. monocytogenes* inoculated on cantaloupe rind samples at 24°C. Low: low inoculum size, High: high inoculum size.

Table 4.11. Cantaloupe rind LS Means Outputs

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT												
i/j	1	2	3	4	5	6	7	8	9	10	11	12
1		0.6406	0.9710	0.3899	0.1196	0.6566	0.1829	0.8329	0.6428	0.2378	0.4552	0.2934
2	0.6406		0.6659	0.6753	0.2265	0.9815	0.0966	0.7948	0.9974	0.4373	0.7673	0.1557
3	0.9710	0.6659		0.4080	0.1256	0.6823	0.1742	0.8612	0.6683	0.2496	0.4756	0.2797
4	0.3899	0.6753	0.4080		0.3931	0.6590	0.0554	0.5044	0.6729	0.7061	0.9002	0.0881
5	0.1196	0.2265	0.1256	0.3931		0.2196	0.0188	0.1593	0.2255	0.6157	0.3352	0.0285
6	0.6566	0.9815	0.6823	0.6590	0.2196		0.0997	0.8125	0.9842	0.4250	0.7499	0.1607
7	0.1829	0.0966	0.1742	0.0554	0.0188	0.0997		0.1374	0.0971	0.0344	0.0652	0.7255

(table cont'd.)

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT												
i/j	1	2	3	4	5	6	7	8	9	10	11	12
8	0.8329	0.7948	0.8612	0.5044	0.1593	0.8125	0.1374		0.7973	0.3143	0.5828	0.2216
9	0.6428	0.9974	0.6683	0.6729	0.2255	0.9842	0.0971	0.7973		0.4355	0.7648	0.1564
10	0.2378	0.4373	0.2496	0.7061	0.6157	0.4250	0.0344	0.3143	0.4355		0.6180	0.0536
11	0.4552	0.7673	0.4756	0.9002	0.3352	0.7499	0.0652	0.5828	0.7648	0.6180		0.1042
12	0.2934	0.1557	0.2797	0.0881	0.0285	0.1607	0.7255	0.2216	0.1564	0.0536	0.1042	

The *Listeria* population declined over time under the storage temperature of 24^{o}C on both high inoculum and low inoculum bell pepper rinds. The initial count on high inoculum samples was 3.51 Log CFU/cm^2, which decreased to 1.60 Log CFU/cm^2 on day 2. In subsequent days, the bacterial count increased with time. On day 14, the bacterial population was 3.11 Log CFU/cm^2 (Figure 4.6). On low inoculum samples, the population slightly decreased from the initial count, 1.86 Log CFU/cm^2 to 1.50 Log CFU/cm^2 after 24 h. While on day 2, count slightly increased to 2.29 Log CFU/cm^2 (Table 4.12). However, in subsequent days, the population decreased with time. On day 14, the level reduced below the detectable limit.

Green Bell Pepper Rind (Low)=-0.14198*DAYS + 1.97004

Green Bell Pepper Rind (High)=-0.02312*DAYS + 2.74930

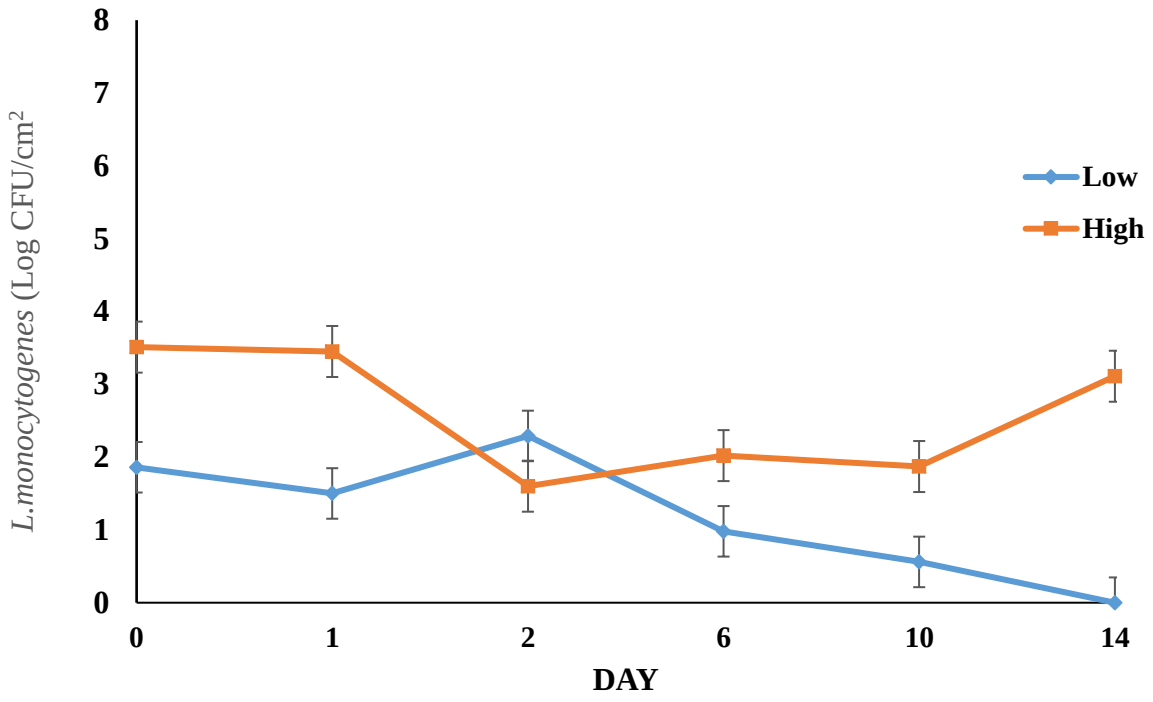

Figure 4.6. Survival of *L. monocytogenes* inoculated on bell pepper rinds at 24°C. Low: low inoculum size, High: high inoculum size.

Table 4.12. Green bell pepper rind LS Means Outputs

Least Squares Means for effect TRT*DAYS Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: COUNT												
i/j	1	2	3	4	5	6	7	8	9	10	11	12
1		0.9577	0.1359	0.2245	0.1878	0.7284	0.1855	0.1206	0.3081	0.0654	0.0393	0.0225
2	0.9577		0.1460	0.2410	0.2017	0.7680	0.1993	0.1295	0.3300	0.0701	0.0420	0.0239
3	0.1359	0.1460		0.7122	0.8118	0.2180	0.8186	0.9295	0.5494	0.5892	0.3654	0.1981

(table cont'd.)

Least Squares Means for effect TRT*DAYS
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: COUNT

i/j	1	2	3	4	5	6	7	8	9	10	11	12
4	0.2245	0.2410	0.7122		0.8945	0.3553	0.8875	0.6492	0.8118	0.3779	0.2245	0.1198
5	0.1878	0.2017	0.8118	0.8945		0.2993	0.9929	0.7448	0.7122	0.4456	0.2680	0.1434
6	0.7284	0.7680	0.2180	0.3553	0.2993		0.2958	0.1935	0.4776	0.1039	0.0613	0.0341
7	0.1855	0.1993	0.8186	0.8875	0.9929	0.2958		0.7514	0.7058	0.4504	0.2711	0.1451
8	0.1206	0.1295	0.9295	0.6492	0.7448	0.1935	0.7514		0.4956	0.6492	0.4084	0.2232
9	0.3081	0.3300	0.5494	0.8118	0.7122	0.4776	0.7058	0.4956		0.2775	0.1627	0.0870
10	0.0654	0.0701	0.5892	0.3779	0.4456	0.1039	0.4504	0.6492	0.2775		0.6930	0.4062
11	0.0393	0.0420	0.3654	0.2245	0.2680	0.0613	0.2711	0.4084	0.1627	0.6930		0.6461
12	0.0225	0.0239	0.1981	0.1198	0.1434	0.0341	0.1451	0.2232	0.0870	0.4062	0.6461	

The contamination of melons and bell peppers with human pathogens have been reported by several studies (Gagliardi, Millner, Lester, & Ingram, 2003; León-Félix, Martínez-Bustillos, Báez-Sañudo, Peraza-Garay, & Chaidez, 2010). The survival of bacterial cells on produce rinds may be influenced by several factors. Steine et al., 2005 observed that cantaloupe surface better supported the survival for microorganisms including *E. coli*, *Shigella* compared to lettuce or bell pepper surfaces indicating produce surface characteristics as an important factor. Our study also observed similar results. An increase in *Listeria* count with time was observed on cantaloupe rinds.

However, there was a decreasing trend in the bacterial population on bell pepper rinds. The study of human pathogens' growth on produce rinds is essential to assess the food produce safety risk during processing of the products. During slicing of produce, pathogens, if present on the rind may transfer to the edible interior. Cantaloupe is a good source of fructose, glucose, and sucrose, with soluble solids ranging from 8 to 14% (Lester & Dunlap, 1985). These nutrients, if leached out on the rind surfaces may support the growth of bacterial pathogens. Furthermore, crevices, cracks may help internalize the pathogens in fruits (Gautam, Dobhal, Payton, Fletcher, & Ma, 2014).

Over all, the study showed that the storage temperature of fruits and vegetables is a critical factor for the survival and growth of *L. monocytogenes*. Storage at refrigerated temperature is a key to reduce produce safety risks. Also, the produce characteristics could be another factor for the survival and growth of human pathogens. The growth of *L. monocytogenes* on cantaloupe rinds at ambient temperature indicated that there is a need for a proper storage strategy for cantaloupe fruits. Further study is needed to understand the interrelationship between comprehensive storage conditions and microbial survival on produce surfaces.

3.5. Conclusions

Fresh-cut fruits stored at 13°C presented a favorable environment for the propagation of *Listeria* monocytogenes. Papaya samples and pears achieved significant increases of 3.80 Log CFU/g and 1.79 Log CFU/g. Likewise, watermelon and cantaloupe samples presented significant increases of 3.45 Log CFU/g and 4.24 Log CFU/g. *Listeria* levels in these samples reached dangerous population levels, considering most samples by the end of analysis were above the infective dose of 4 Log CFU/g for immunocompromised individuals. These surfaces present exposed areas in which pathogens such as *Listeria monocytogenes* may effectively attach. Considering also that these fruits are rich in nutrients, the pathogen not only attaches but also can multiply significantly throughout the shelf life of these products. Pineapple, despite being rich in nutrients as the other fruits sampled, proved to be an unfavorable environment for *Listeria monocytogenes*. This emphasizes the importance of microbial barriers, such as acidic pH levels in minimally processed products, in order to control pathogenic contamination.

Rind samples exposed two major issues concerning the attachment of *Listeria monocytogenes* on the external surfaces of cantaloupes and green bell peppers. Cantaloupe surfaces are ideal for the attachment of *Listeria monocytogenes*, but the lack of nutrients in the surface of intact cantaloupe rinds makes it difficult for microbial populations to increase above the 4 Log CFU/cm^2 threshold. If these surfaces presented injuries, it would alter the behavior of this bacteria because it could expose the nutrients present in the cantaloupe flesh, and as proven by the fresh-cut study this is a favorable environment for the growth of *Listeria monocytogenes*. Green bell peppers, on the other hand, presented a surface difficult for attachment of *Listeria monocytogenes*, due to their impermeability. This difficulty leads to a decrease in low inoculum bacteria levels.

However, this study was able to show that at higher inoculum levels the pathogen manages to survive during the shelf life of the product.

Upon completion of this study, two more projects were planned out as future work, both of them concerning the search of efficient methods of controlling pathogenic contamination in fresh produce. The first study is related to the use of clamshell packaging and the incorporation of antimicrobial films on these packages, in order to control and reduce pathogens that may have contaminated the surface. The second study is related to the use of gaseous chlorine dioxide as a method of reducing microbial load on fresh produce after contamination.

APPENDIX SAS COMMAND INPUTS

Fresh-cut Pear:

```
DATA DIEOFF PEAR FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.203160577
4°C 1 1 3.374558187
4°C 1 2 3.337497448
4°C 1 4 3.475216156
4°C 1 6 3.512639481
4°C 2 0 3.286435801
4°C 2 1 3.373919664
4°C 2 2 3.346285159
4°C 2 4 3.687840948
4°C 2 6 3.499985098
13°C 1 0 3.338951661
13°C 1 1 3.861185182
13°C 1 2 4.515670787
13°C 1 4 5.139724535
13°C 1 6 5.141008304
13°C 2 0 3.423224831
13°C 2 1 3.128872213
13°C 2 2 4.21367161
13°C 2 4 4.625412933
13°C 2 6 5.20625241
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Papaya:

```
DATA DIEOFF PAPAYA FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 2.839720095
4°C 1 1 3.050178544
4°C 1 2 3.137626034
4°C 1 4 2.975863949
4°C 1 6 3.487893715
4°C 2 0 3.126880933
```

```
4°C 2 1 3.365516249
4°C 2 2 3.382685199
4°C 2 4 3.684309908
4°C 2 6 3.597543073
13°C 1 0 3.248453077
13°C 1 1 3.892572279
13°C 1 2 4.874506942
13°C 1 4 6.608558055
13°C 1 6 7.573233079
13°C 2 0 3.599454669
13°C 2 1 3.960627877
13°C 2 2 5.27413442
13°C 2 4 6.69875326
13°C 2 6 6.87440013
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Pineapple:

```
DATA DIEOFF PINEAPPLE FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.159031667
4°C 1 1 3.032204322
4°C 1 2 2.612971322
4°C 1 4 0.000000001
4°C 1 6 1.751161473
4°C 2 0 3.380116973
4°C 2 1 3.509544528
4°C 2 2 3.378086403
4°C 2 4 3.135190866
4°C 2 6 2.502566083
13°C 1 0 3.337247575
13°C 1 1 3.124032863
13°C 1 2 2.717250932
13°C 1 4 1.659784008
13°C 1 6 1.573537825
13°C 2 0 3.549709685
13°C 2 1 3.222039543
13°C 2 2 1.601162088
13°C 2 4 1.641617267
13°C 2 6 1.716173896
```

```
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Watermelon:

```
DATA DIEOFF WATERMELON FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C    1    0    3.340620619
4°C    1    1    3.228970901
4°C    1    2    3.66701271
4°C    1    4    3.557977339
4°C    1    6    3.852810172
4°C    2    0    3.263925695
4°C    2    1    3.526798356
4°C    2    2    3.584280636
4°C    2    4    3.955355328
4°C    2    6    3.880858444
13°C   1    0    3.441705126
13°C   1    1    4.541139264
13°C   1    2    5.858736513
13°C   1    4    6.712049914
13°C   1    6    6.818638455
13°C   2    0    3.700115803
13°C   2    1    4.813588065
13°C   2    2    6.307750019
13°C   2    4    6.518717753
13°C   2    6    7.220802922
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Cantaloupe:

```
DATA DIEOFF CANTALOUPE FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C    1    0    3.041392685
```

```
4°C 1 1 3.322219295
4°C 1 2 3.243038049
4°C 1 4 3.342422681
4°C 1 6 3.574031268
4°C 2 0 3.371067862
4°C 2 1 4.204119983
4°C 2 2 3.06069784
4°C 2 4 3.397940009
4°C 2 6 3.361727836
13°C 1 0 3.417284915
13°C 1 1 5.036371295
13°C 1 2 6.223318752
13°C 1 4 7.111261875
13°C 1 6 7.623634031
13°C 2 0 3.456545113
13°C 2 1 4.866147978
13°C 2 2 6.125397542
13°C 2 4 6.729397366
13°C 2 6 7.736916433
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Broccoli:

```
DATA DIEOFF BROCCOLI FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.391709082
4°C 1 1 3.003511934
4°C 1 2 3.002286473
4°C 1 4 2.575353819
4°C 1 6 2.494734582
4°C 2 0 3.310441945
4°C 2 1 2.867781503
4°C 2 2 2.483582074
4°C 2 4 2.554769627
4°C 2 6 2.595713643
13°C 1 0 3.196428718
13°C 1 1 3.24827914
13°C 1 2 2.941860692
13°C 1 4 2.481919414
13°C 1 6 2.75187555
```

```
13°C 2 0 3.356232428
13°C 2 1 3.472687696
13°C 2 2 3.145528684
13°C 2 4 3.434547003
13°C 2 6 3.119929077
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Cauliflower:

```
DATA DIEOFF CAULIFLOWER FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.325493077
4°C 1 1 2.725205723
4°C 1 2 2.916198426
4°C 1 4 2.535624547
4°C 1 6 2.174873254
4°C 2 0 3.279700732
4°C 2 1 2.994472751
4°C 2 2 3.209730909
4°C 2 4 2.010283347
4°C 2 6 2.069657534
13°C 1 0 3.190411771
13°C 1 1 3.611339702
13°C 1 2 2.32941729
13°C 1 4 3.354163427
13°C 1 6 3.71816178
13°C 2 0 3.560131765
13°C 2 1 3.576742932
13°C 2 2 3.369763333
13°C 2 4 3.274038919
13°C 2 6 3.766191632
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Lettuce:

```
DATA DIEOFF LETTUCE FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.320860672
4°C 1 1 2.509336234
4°C 1 2 2.619805106
4°C 1 4 2.75444268
4°C 1 6 2.329612588
4°C 2 0 3.337024331
4°C 2 1 3.328953007
4°C 2 2 3.021659469
4°C 2 4 3.95034986
4°C 2 6 3.484375107
13°C 1 0 3.321646863
13°C 1 1 3.53508311
13°C 1 2 3.130801966
13°C 1 4 3.514529475
13°C 1 6 4.175528747
13°C 2 0 3.403135098
13°C 2 1 3.295742114
13°C 2 2 3.331775567
13°C 2 4 3.627031107
13°C 2 6 4.191836866
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Kale:

```
DATA DIEOFF KALE FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C 1 0 3.103930706
4°C 1 1 2.951938217
4°C 1 2 2.673933226
4°C 1 4 2.401552366
4°C 1 6 2.634308485
4°C 2 0 4.342370959
4°C 2 1 3.737261371
4°C 2 2 3.68944352
4°C 2 4 3.442127141
4°C 2 6 3.352777918
```

```
13°C  1  0  3.950556905
13°C  1  1  4.764697851
13°C  1  2  3.738222404
13°C  1  4  3.604425706
13°C  1  6  4.294511523
13°C  2  0  4.577762673
13°C  2  1  4.948816294
13°C  2  2  4.918055213
13°C  2  4  5.317630484
13°C  2  6  4.357381919
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Fresh-cut Green Bell Pepper:

```
DATA DIEOFF BELLPEPPER FC;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
4°C  1  0  3.06069784
4°C  1  1  2.397940009
4°C  1  2  2.84509804
4°C  1  4  3.146128036
4°C  1  6  3.414973348
4°C  2  0  3.146128036
4°C  2  1  3.176091259
4°C  2  2  2.77815125
4°C  2  4  2.602059991
4°C  2  6  2.84509804
13°C  1  0  3.265819758
13°C  1  1  3.086740785
13°C  1  2  4.472742746
13°C  1  4  5.272194674
13°C  1  6  5.320785873
13°C  2  0  3.59495632
13°C  2  1  4.089214258
13°C  2  2  4.746235335
13°C  2  4  5.030931967
13°C  2  6  4.343881866
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
```

```
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;

DATA DIEOFF BP R;
INPUT TRT BLK DAYS COUNT;
DATALINES;
1       1       0       2.37
1       1       1       1.65
1       1       2       3.53
1       1       6       1.95
1       1       10      1.05
1       1       14      0.00
1       2       0       1.34
1       2       1       1.34
1       2       2       1.04
1       2       6       0.00
1       2       10      0.00
1       2       14      0.00
2       1       0       3.66
2       1       1       3.54
2       1       2       1.53
2       1       6       4.03
2       1       10      3.73
2       1       14      4.41
2       2       0       3.35
2       2       1       3.35
2       2       2       1.66
2       2       6       0.00
2       2       10      0.00
2       2       14      1.81
;
PROC GLM;
CLASS TRT BLK DAYS;
MODEL COUNT=TRT BLK DAYS TRT*DAYS TRT*BLK BLK*DAYS;
LSMEANS TRT*DAYS TRT*BLK BLK*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

```
DATA DIEOFF BP R;
INPUT TRT BLK DAYS COUNT;
DATALINES;
1	1	0	2.37
1	1	1	1.65
1	1	2	3.53
1	1	6	1.95
1	1	10	1.05
1	1	14	0.00
1	2	0	1.34
1	2	1	1.34
1	2	2	1.04
1	2	6	0.00
1	2	10	0.00
1	2	14	0.00
2	1	0	3.66
2	1	1	3.54
2	1	2	1.53
2	1	6	4.03
2	1	10	3.73
2	1	14	4.41
2	2	0	3.35
2	2	1	3.35
2	2	2	1.66
2	2	6	0.00
2	2	10	0.00
2	2	14	1.81
;
PROC SORT; BY DAYS;
PROC GLM; BY DAYS;
CLASS TRT BLK;
MODEL COUNT=TRT BLK;
MEANS TRT/TUKEY;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

Cantaloupe Rind:

```
DATA DIEOFF C RIND;
INPUT TRT $ REP DAYS COUNT;
DATALINES;
low     1       0       2.05
low     1       1       4.65
low     1       2       5.17
low     1       4       6.44
low     1       6       3.33
low     1       8       1.03
low     2       0       2.41
low     2       1       4.90
low     2       2       5.16
low     2       4       6.33
low     2       6       7.91
low     2       8       4.50
high    1       0       4.26
high    1       1       5.21
high    1       2       5.68
high    1       4       4.40
high    1       6       6.68
high    1       8       5.95
high    2       0       4.65
high    2       1       5.13
high    2       2       3.34
high    2       4       7.22
high    2       6       7.63
high    2       8   4.32
;
PROC GLM;
CLASS TRT REP DAYS;
MODEL COUNT=TRT REP DAYS TRT*DAYS TRT*REP REP*DAYS;
LSMEANS TRT*DAYS TRT*REP REP*DAYS/STDERR PDIFF;
ODS HTML CLOSE;
ODS HTML;
RUN;
```

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